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MINIREVIEW

Viral and Host Factors in Human Respiratory Syncytial Virus Pathogenesis▽

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Human respiratory syncytial virus (RSV) was first isolated in 1956 from a laboratory chimpanzee with upper respiratory tract disease (for general reviews, see references 21, 57, 102, and 145). RSV was quickly determined to be of human origin and was shown to be the leading worldwide viral agent of serious pediatric respiratory tract disease. In a 13-year prospective study of infants and children in the United States, RSV was detected in 43%, 25%, 11%, and 10% of pediatric hospitalizations for bronchiolitis, pneumonia, bronchitis, and croup, respectively (110). Approximately two-thirds of infants are infected with RSV during the first year of life, and 90% have been infected one or more times by 2 years of age. The rate of hospitalization for primary infection is approximately 0.5% but can vary by situation and ethnic group and can be as high as 25% (77).

RSV also is a significant cause of morbidity and mortality in the elderly, with an impact approaching that of nonpandemic influenza virus (39). RSV readily infects severely immunocompromised individuals, most notably allogeneic bone marrow transplant recipients, causing high mortality. RSV also makes a substantial contribution to upper respiratory tract disease in individuals of all ages (59, 65). Globally, the World Health Organization estimates that RSV causes 64 million infections and 160,000 deaths annually (Initiative for Vaccine Research: respiratory syncytial virus, World Health Organization [http://www.who.int/vaccine_research/diseases/ari/en/index3.html, accessed 5 December 2005]).

Although RSV has a single serotype, reinfection can occur throughout life. RSV in yearly winter/early spring epidemics in temperate regions; elsewhere, the timing of RSV activity can vary widely with the locale. The RSV reservoir in the off-season is unknown. Strains circulate quickly around the earth (150). Neither a vaccine nor an effective antiviral therapy is available, although there is active research in both areas (23, 78, 138). However, infants at high risk for serious disease can receive passive immunoprophylaxis during the epidemic season by a monthly injection of a commercial RSV-neutralizing monoclonal antibody, palivizumab (Synagis), which provides a 55% reduction in RSV-associated hospitalization (17).

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THE VIRUS

RSV (family *Paramyxoviridae*, order *Mononegavirales*) is an enveloped virus with a single-stranded negative-sense RNA genome of 15.2 kb (21). There are animal versions of RSV, including bovine RSV (BRSV) and pneumonia virus of mice (PVM), suggesting that species jumping occurred during the evolution of these viruses. However, there is no animal reservoir for human RSV.

Efficient infection by RSV of established cell lines in vitro involves binding to cell-surface glycosaminoglycans (62). However, it is not known how closely this binding models attachment in vivo or whether it is an initial interaction that is followed by a second, higher-affinity step that remains to be identified. The nucleocapsid gains entry to the cytoplasm by membrane fusion; surprisingly, this may involve clathrin-mediated endocytosis rather than surface fusion typical of paramyxoviruses (85). Viral gene expression and RNA replication occur in the cytoplasm, and virions acquire a lipid envelope by budding through the plasmid membrane (Fig. 1). Virions are pleomorphic and include spheres and long, fragile filaments. Studies with RSV are impeded by modest viral yields in cell culture and physical instability of the particle; interestingly, this instability may reside in the glycoproteins (133).

The negative-sense RNA genome contains a short 3′-extragenic leader region, 10 viral genes in a linear array, and a 5′-trailer region (Fig. 1). Each gene is transcribed into a separate, capped, polyadenylated mRNA encoding a single viral protein, except in the case of the M2 mRNA, which contains two overlapping open reading frames that are expressed by a ribosomal stop-restart mechanism into two distinct proteins, M2-1 and M2-2 (52).

Five RSV proteins are involved in nucleocapsid structure and/or RNA synthesis (21). The nucleocapsid N protein tightly encapsidates genomic RNA as well as its positive-sense replicative intermediate, called the antigenome. This provides protected, flexible templates and probably reduces detection of these viral RNAs by host cell toll-like receptors (TLRs) and intracellular RNA recognition helicases that initiate innate immune responses through interferon (IFN) regulatory factors and nuclear factor κB (NF-κB) (3, 94). The large L protein is the major polymerase subunit and contains the catalytic domains. The P phosphoprotein is an essential cofactor in RNA synthesis (31) and also is thought to associate with free N and L to maintain them in soluble form for assembly of and interaction with nucleocapsids. The M2-1 and M2-2 proteins are

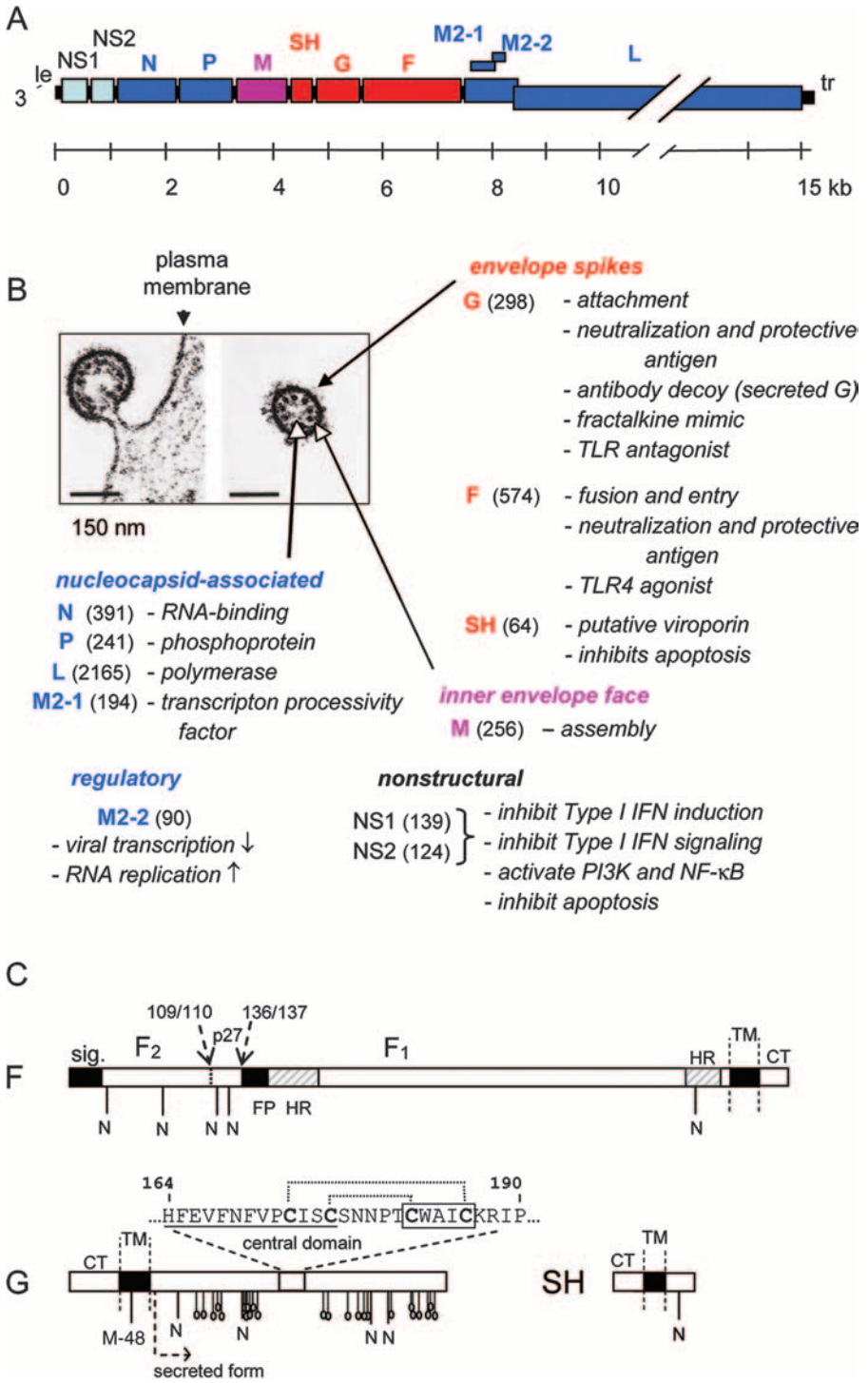

FIG. 1. RSV virion, RNA genome, and encoded proteins. (A) The negative-sense RNA genome (strain A2) is depicted 3′ to 5′ showing the extragenic 3′ leader (le) and 5′ trailer (tr) regions and the intervening 10 viral genes (rectangles) that are each expressed as a separate mRNA (21). M2-1 and M2-2 are overlapping open reading frames of the M2 mRNA. The M2 and L genes overlap slightly, and L is expressed by polymerase backtracking (25). (B) Electron photomicrographs showing an RSV virion budding through the plasma membrane of an infected cell (left) and a free virion (right). Protein functions and amino acid lengths (in parentheses, unmodified form) are indicated. (C) Schematic diagrams of the F, G, and SH proteins, with lengths approximately to scale (21). Filled rectangles indicate the hydrophobic cleaved signal sequence (sig.), transmembrane anchors (TM), and fusion peptide (FP). Cross-hatched rectangles indicate heptad repeats (HR) that drive conformational changes involved in fusion. CT, cytoplasmic domain. Potential acceptor sites for N-linked sugars are indicated as downward-facing stalks with an N. For the F protein, the locations and amino acid positions of the two cleavage sites are indicated, as are the cleavage products (F_2, p27, F_1). For the G protein, the 25 potential acceptor sites for O-linked sugars predicted to be the most likely to be utilized are indicated as downward-facing lollipops. The sequence and disulfide-bonding pattern (dotted lines) of the central domain are shown with the CX3C fractalkine motif boxed and the highly conserved 13-amino-acid sequence of unknown significance underlined. The M-48 translational start site for the secreted form and the mature secreted form is indicated.

factors involved, respectively, in transcription (42) and in modulating the balance between transcription and RNA replication (9).

Four other RSV proteins associate with the lipid bilayer to form the viral envelope (21). The matrix M protein lines the inner envelope surface and is important in virion morphogenesis (147). The heavily glycosylated G, fusion F, and small hydrophobic SH proteins are transmembrane surface glycoproteins (Fig. 1). G and F are the only virus neutralization antigens and are the two major protective antigens (21).

The G glycoprotein plays a major but not exclusive role in viral attachment (148). It contains several N-linked carbohydrate side chains and an estimated 24 to 25 O-linked side chains. This increases the apparent molecular weight of the polypeptide backbone from 32,500 to 90,000. Most of the ectodomain is thought to have an extended, unfolded, heavily glycosylated, mucin-like structure that is unique to RSV and its close relatives and appears to be very distinct from the globular attachment proteins of other paramyxoviruses. The significance of the similarity to mucin is unknown, although it is tempting to speculate that it might alter the physicochemical properties of the virus so as to facilitate spread or evade trapping by mucus. G is anchored in the membrane by a signal/anchor sequence near the N terminus and also is expressed as a secreted form. This secreted form arises from translational initiation at the second methionine (codon 48) in the open reading frame followed by proteolytic trimming to yield a final form that lacks the N-terminal 65 amino acids, including the entire signal/anchor (129). The G ectodomain contains a highly conserved domain of 13 amino acids whose significance is unknown (146). This conserved sequence overlaps a disulfide-bonded tight turn that is called a cystine noose and contains a CX3C motif that is discussed later.

The F protein directs viral penetration by membrane fusion and also mediates fusion of infected cells with their neighbors to form syncytia. F is synthesized as a precursor, F_0, which is activated by cleavage by furin-like intracellular host protease. Unusual for a viral penetration protein, cleavage occurs at two sites (amino acids 109/110 and 136/137) (Fig. 1) (51). This yields, in amino-to-carboxy-terminal order, F_2 (109 amino acids), p27 (27 amino acids), and F_1 (438 amino acids). F_2 and F_1 remain linked by a disulfide bond and represent the active form.

The remaining two RSV proteins, NS1 and NS2, are small species that do not appear to be packaged significantly in the virion. As described below, they are nonessential accessory proteins involved in modulating the host response to infection.

Gene expression and RNA replication by RSV broadly follow the mononegavirus model, although admittedly there are substantial gaps in our understanding of these processes even for prototypical mononegavirues (25). The polymerase enters the genome at or near its 3′ end, and the genes are transcribed into individual mRNAs by sequential start-stop-restart synthesis that is guided by short transcription signals flanking the genes. There is a polar gradient of mRNA abundance due to polymerase fall-off. RNA replication involves synthesis of the full-length positive-sense antigenome that in turn is copied into progeny genomes.

RSV adds some complexity of its own with the M2-1 and M2-2 proteins, which are found only in close relatives of RSV. With RSV, processive transcription depends on the M2-1 protein, which is essential for viral viability (42). In its absence, transcription terminates nonspecifically within several hundred nucleotides and results in (reduced) expression of NS1 and NS2 alone (42). It is tempting to speculate that a reduction in the level of M2-1 might facilitate persistent infection by downregulating the expression of most of the viral genes while maintaining some expression of the NS1 and NS2 host defense antagonists. The other product of the M2 gene, the M2-2 protein, is not essential but appears to downregulate transcription in favor of RNA replication as infection progresses (9). It is unclear why RSV needs these extra proteins while other mononegaviruses, which seem to have a very similar RNA synthetic program, do not. Interestingly, the M2-1 protein of human metapneumovirus (HMPV) shares substantial sequence identity with that of RSV but is not essential for processive transcription or viral viability (15). It may be that there are other M2-1 functions that remain to be identified.

CLINICAL INFECTION AND DISEASE

Inoculation of the nose or eyes occurs by large particle aerosol or direct contact and results in viral replication in the nasopharynx, with an incubation period of 4 to 5 days, and can be followed over the next several days by spread to the lower respiratory tract (21, 57, 102). Rhinorrhea, cough, and low-grade fever are common. Signs of lower airway infection are common even in infants with mild disease. Clinical signs of bronchiolitis include increased airway resistance, air trapping, and wheezing. Pneumonia accounts for the hypoxia frequently detected in RSV-infected infants.

Infection normally is highly restricted to the superficial cells of the respiratory epithelium (72, 159). Ciliated cells of the small bronchioles and type 1 pneumocytes in the alveoli are major targets of infection in the lower airway. It is likely that other cells, including nonciliated epithelium and intraepithelial dendritic cells (DCs), are also infected (Fig. 2), but the basal cells appear to be spared (72). Pathological findings include necrosis of epithelial cells, occasional proliferation of the bronchiolar epithelium, infiltrates of monocytes and T cells centered on bronchiolar and pulmonary arterioles, and neutrophils between vascular structures and small airways. Infection and tissue damage tends to be patchy rather than diffuse. There are abundant signs of airway obstruction due to sloughing of epithelial cells, mucus secretion, and accumulated immune cells. Syncytia are sometimes observed in the bronchiolar epithelium but are not common. However, syncytium formation and giant-cell pneumonia are hallmarks of infection in individuals with extreme T-cell deficiency.

Fifty percent or more of infants hospitalized with RSV lower respiratory tract disease have subsequent episodes of wheezing that in some cases can persist until 11 years of age or more (57, 140). It is of interest whether infection is a causal factor or whether severe infection and wheezing are comarkers of an underlying vulnerability. Evidence for causality in at least a subset of individuals comes from a recent study in which successful palivizumab prophylaxis of preterm infants was associated with reduced wheezing compared to untreated controls when assessed at approximately 3.5 years of age (140). There also is evidence that congenital vulnerability is involved and an

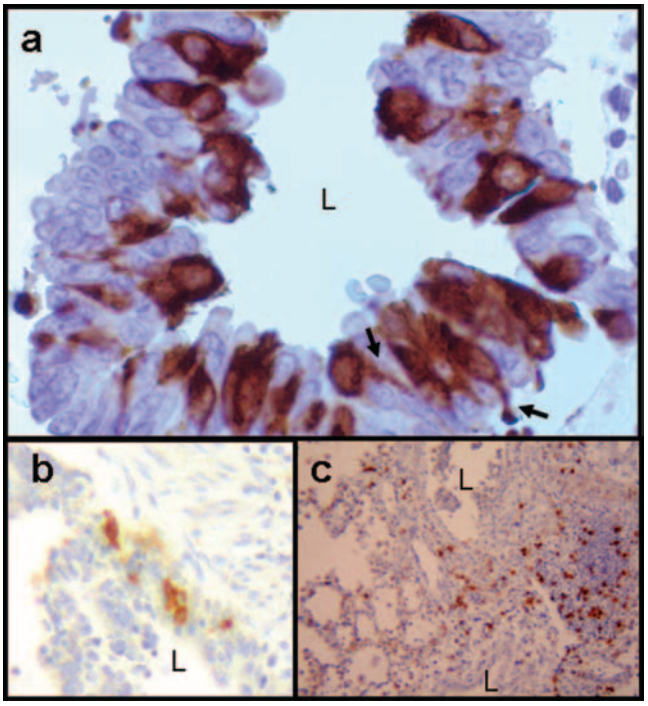

FIG. 2. Immunohistochemistry of autopsy lung specimens from a 15-month-old patient with an untreated RSV infection (72). Immunostaining for RSV antigens (a) revealed infection of the bronchiolar epithelium in a near circumferential pattern sparing the basal cells. The basal cells can restore the epithelium, but in the process may lead to mucus metaplasia and remodeling of airways. In addition to infection of the polarized ciliated epithelium, it appears that other cell types, potentially including intraepithelial DCs, can be infected (arrows point to the projections of irregularly shaped RSV-infected cells that are thought to be DCs, based on morphology). Immunostaining for $CD1a^+$ DCs (b) showed that they are not prominent in the lung parenchyma during infection, but when present can have a distinctive morphology with multiple projections that can extend beyond the apical or basal boundary of the columnar epithelium. $CD69^+$ monocytes were the major cell type present in peribronchiolar infiltrates. However, $CD3^+$ T cells also were abundant and, while most of them appeared to be double negative for CD4 and CD8, many were positive for CD8 immunostaining (c). In panels a to c, L indicates airway lumen; in panel c, the lower lumen is occluded with debris and immune cells.

indication that there is considerable host-dependent heterogeneity in long-term effects of infection (98, 99). Some studies also have linked severe early RSV infection with allergic sensitization leading to asthma, but other researchers have disputed this link (36, 139). In a small study, antibody prophylaxis against RSV was associated with a higher incidence of normal lung function and lower incidence of atopy compared with controls when evaluated 7 to 10 years later (160).

There presently is renewed interest in whether RSV can establish persistent infection in vivo, which might contribute to pathogenesis and also help maintain the virus in the population. In mice previously infected with, and apparently cleared of, RSV, suppression of T cells several months later resulted in the emergence of infectious virus in some animals (136). An analysis of patients with chronic obstructive pulmonary disease provided evidence of long-term infection in one study (162); however, a second study with a comparable patient group indicated that RSV was present in acute rather than persistent infections (38).

The following sections will outline host risk factors for severe RSV disease, the protective immune response and possible deficiencies, the contribution of host immunity to pathogenesis, and viral factors in pathogenesis. Figure 3 depicts a model of the relative contributions of host versus viral factors to pathogenesis versus protection.

HOST RISK FACTORS

The risk of severe RSV disease is increased by factors that compromise the ability to control and withstand a respiratory tract infection: young age (<6 months), premature birth (<35 weeks of gestation), bronchopulmonary dysplasia, congenital heart disease, immunodeficiency or immunosuppression, the first or second RSV infection in life, unusually narrow airways, low birth weight, male gender, a low titer of RSV-specific serum antibodies, and frail old age (157). Although prematurity and underlying disease play important roles in pediatric RSV disease, more than two-thirds of pediatric hospitalizations involve previously healthy infants. Environmental factors, including ones that affect lung function (e.g., household tobacco use) or that increase exposure to infection (e.g., day care, hospitalization, multiple siblings), also play a role.

A role for genetic predisposition in severe RSV disease is indicated by (i) association of susceptibility with a family history of asthma or severe infant lower respiratory tract disease and (ii) differences in susceptibility between ethnic, racial, and gender groups (69). More recently, studies have provided evidence associating increased incidence of severe pediatric RSV disease with genetic polymorphisms in a number of genes selected for analysis, although these studies seem preliminary and sometimes are inconsistent. These associations include genes encoding cytokines and chemokines, including interleukin-4 (IL-4), IL-8/CXCL8, IL-10, IL-13, and RANTES/CCL5, or encoding proteins involved in surface interactions or intracellular signaling, such as TLR4, CD14, IL-4R, CX3CR1, CCR5, and surfactant protein A (SP-A), SP-B, SP-C, and SP-D (4, 6, 69, 71, 115, 125–127, 144). Some of these polymorphisms have been associated with functional effects that give clues to possible beneficial or pathological roles of host factors in RSV infection, some of which are noted below.

PROTECTIVE AND PATHOGENIC FEATURES OF THE HOST RESPONSE

Protective immunity. As with other acute respiratory viruses, RSV infection usually is completely resolved by innate and adaptive immunity (21, 57, 102, 145). As with many viruses, RSV infection or uptake by respiratory epithelial cells and resident macrophages results in widespread changes in cellular gene expression and upregulates expression of a variety of factors, including surfactants, cytokines, chemokines, and cell-surface molecules. Some of these factors have direct antiviral properties; others stimulate the influx and activation of natural killer (NK) cells, granulocytes, monocytes, macrophages, dendritic cells, and T lymphocytes that provide direct antiviral activities and initiate an effective adaptive immune response.

Virus-neutralizing antibodies in the respiratory tract likely

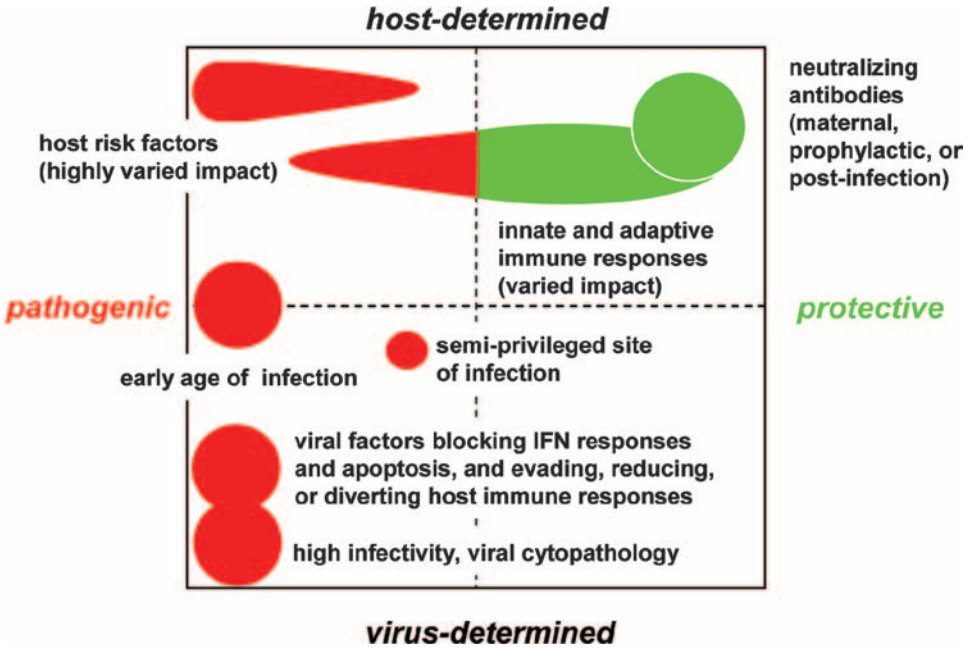

FIG. 3. Estimated contributions of host and viral factors to RSV pathogenesis in the overall pediatric population, as discussed in the text. Factors are placed in the vertical dimension approximately according to the extent to which they are determined by the host (top) or virus (bottom) or a combination (in between). Placement in the horizontal dimension indicates the extent to which they are pathogenic (left) or protective (right). The size of the symbol represents speculated aggregate impact.

contribute to viral clearance and certainly play an important role in protection against reinfection (21). They include secretory immunoglobulin A (IgA) and transudated, serum-derived IgG. Secretory IgA is particularly important in protecting the upper respiratory tract, which is accessed only very inefficiently by serum IgG (124, 137). The IgA response is short-lived following primary infection but can increase in duration following reinfection (109). Serum IgG antibodies are somewhat more efficient in accessing the lower respiratory tract and can provide substantial protection in that compartment. In RSV-naïve infants, the maternal serum antibody titer is positively correlated with a reduced level of severe RSV disease. The clinical experience with palivizumab also shows that serum antibodies alone can provide substantial protection from severe disease. However, protection from passive antibodies quickly wanes, because they decay with a half-life of approximately 21 to 24 days. $CD8^+$ T lymphocytes are important for clearing virus-infected cells as well as for contributing cytokines, notably gamma IFN (IFN-γ), that promote a protective Th1 response (53, 142).

Protective immunity to RSV induced by natural infection is generally described in the literature as weak and short-lived. This is based mainly on the frequent incidence of reinfection of humans in nature and under experimental conditions. However, as discussed later, viral immune evasion strategies also may contribute to reinfection. Typical RSV-neutralizing serum antibody titers in adults are quite high (mean reciprocal titer of 1,450 in a 50%-infected-well-reduction assay), and following natural infection, they were increased fourfold or more in 64% of young adult and 79% of frail elderly patients, suggestive of good responses (41). While postinfection increases in antibody titers wane in most individuals within a year, this decay might not be unique to RSV (40), and the residual titers remain quite high. Brisk serum antibody responses also have been noted in children with primary and secondary infections (66), and even young infants of 2 months of age can have substantial neutralizing serum antibody responses when the titer of immunosuppressive maternal antibody is low (R. A. Karron, personal communication). Primary RSV infection of seronegative experimental animals, including the chimpanzee, results in robust protective immune responses, at least in the short term (23). In addition, in clinical studies, experimental live RSV vaccines do not seem to be obviously reduced in immunogenicity compared to live human parainfluenza virus type 3 (HPIV3) and influenza A virus vaccines, although these studies were not designed for virus-to-virus comparisons (R. A. Karron, personal communication). There are reports of effects on cellular immunity. RSV-specific T-helper cell responses, as measured by in vitro lymphoproliferation, appeared to be deficient during reinfection in infancy (12). Increased apoptosis of $CD4^+$ and $CD8^+$ lymphocytes resulting in lymphopenia also has been described for RSV-infected infants compared to uninfected controls, with the effect being greater with younger age and more severe illness (130). Mitogen-induced proliferation of peripheral blood lymphocytes in vitro was inhibited by contact with RSV-infected cell monolayers, an effect that did not prevent the expression of T-cell activation markers but impeded the cell cycle (135). This effect appeared to be mediated by the viral F protein and was augmented by G. Studies of mice suggested that the pulmonary $CD8^+$ CTL response to RSV was less functional and shorter lived than that to influenza virus (18). However, this difference between viruses has not been confirmed and, as noted later, functional impairment to the pulmonary CTL response might be a feature of the

tissue rather than the virus (153). In summary, it remains unclear to what extent frequent reinfection by RSV reflects inadequate or inappropriate responses by the host versus viral immune evasion strategies.

Contribution of the host response to pathogenesis. The most dramatic demonstration of the potential of host immunity to contribute to disease associated with an RSV infection was the experience with a formalin-inactivated RSV (FI-RSV) vaccine that was administered intramuscularly to infants and children in the 1960s. This vaccine was poorly protective and, in RSV-naïve individuals, it primed for enhanced disease upon subsequent natural infection with RSV, with up to 80% of vaccinees hospitalized with RSV-like disease, resulting in two deaths (21, 123). Retrospective studies showed that FI-RSV induced serum antibodies that bound efficiently to viral antigen but did not efficiently neutralize infectivity, contributing to the poor vaccine efficacy (111). This atypical antibody response probably reflected denaturation of the antigen as well as a possible deficiency in antibody affinity maturation. An analysis of lung tissue from the fatal vaccine cases and from experimental animal models of enchanced disease provided evidence of antibody-antigen complex deposition and complement activation in the lung occurring during subsequent RSV infection (120).

In addition, peripheral blood lymphocytes from FI-RSV vaccinees exhibited an exaggerated proliferative response to RSV antigens in vitro compared to lymphocytes isolated following natural infection (82). Subsequent studies with experimental animals confirmed that, compared to natural infection, FI-RSV induced a heightened response of virus-specific $CD4^+$ T lymphocytes biased toward the Th2 subset (54). Th1 and Th2 $CD4^+$ T lymphocytes play important roles in immune regulation and function and to some extent are self-stimulatory and reciprocally inhibitory. Th1 responses (signature cytokines IFN-γ and IL-12) tend to promote cell-mediated immunity important for protection against intracellular pathogens, such as viruses. Th2 responses (signature cytokines IL-4, IL-5, IL-10, and IL-13) can be associated with eosinophilia, goblet cell hyperplasia, mucus overproduction, IgE production, and airway hypersensitivity. The Th2-biased response to FI-RSV appears to be a consequence of inefficient induction of IFN-γ-secreting NK cells and $CD8^+$ T lymphocytes by this nonreplicating vaccine (70, 74). In FI-RSV-immunized animals, depletion studies confirmed that the Th2 cells and cytokines were important in vaccine-enhanced pathology (24). This experience amply demonstrated immune-mediated (particularly Th2-mediated) pathology associated with an inactivated vaccine and subsequent RSV infection. However, comparable disease enhancement does not occur with natural RSV infections and reinfections, and the relevance of FI-RSV-associated pathogenesis to natural infection is unclear.

As often is the case for acute infections, host immunity appears to contribute to pathogenesis during natural RSV infection, although not as dramatically as with FI-RSV. However, the relative contributions to RSV pathogenesis of direct viral cytopathology versus the host immune response, and the host factors that are responsible, remain controversial.

Several observations suggest a substantial contribution of host immunity to RSV disease. For example, clinical observations (as noted above) and in vitro studies (described later) showed that RSV is not highly cytopathic or invasive. In infants coinfected with human immunodeficiency virus type 1, prolonged clinical shedding occurred for more than 199 days without substantial disease (83). When cotton rats with an established RSV infection were administered a neutralizing antibody that reduced pulmonary virus replication more than 1,000-fold, there was little effect on pulmonary pathology; the addition of anti-inflammatory glucocorticoid therapy was necessary to reduce pathology (122). A similar lack of clinical improvement was observed for intubated children with an established infection, for whom antibody therapy reduced viral shedding 30-fold compared to controls (97). The inability to block disease progression by sharply reducing virus replication is suggestive of immunopathology rather than direct viral cytopathology. Finally, genetic polymorphisms that increase expression of the IL-4, IL-8, and (tentatively) CCR5 genes were associated with an increased frequency of severe pediatric RSV disease, suggesting that these host factors can contribute to pathogenesis (69).

Conversely, other observations indicate a contribution of viral cytopathology to RSV disease. RSV disease often is more frequent and more severe in highly immunosuppressed or immunocompromised individuals, which seems inconsistent with disease being primarily immune mediated (43). In humans, immunity to RSV due to maternal antibodies or prior infection ameliorates rather than enhances disease upon reinfection. While it is not as cytopathic as influenza virus, RSV perturbs ciliary action and leads to cell shedding, effects that would contribute to airway obstruction. There generally is a positive correlation between the magnitude of virus replication and clinical disease in natural and experimental infections with wild-type or attenuated RSV (30, 79), although findings to the contrary also have been reported (163). However, this does not argue solely for viral cytopathology, since diminishing the viral load will reduce the antigenic stimulation driving immunopathology. In experimental infections of chimpanzees and human adults, children, and infants with wild-type or attenuated RSV, symptoms began 1 to 4 days following the onset of viral shedding and ended either coincident with the cessation of shedding or continued for several days, depending on the individual (8, 75, 79, 81, 128). This result suggests that both viral cytopathology and host immunity contribute to disease and that there is variation among different individuals. A recent evaluation of lung specimens from young infants with rapidly fatal, untreated RSV infection provided evidence of extensive viral replication with few $CD8^+$ T lymphocytes or NK cells and minimal lymphocyte-derived cytokines (159). This suggested that these cases of fatal disease involved an inadequate rather than excessive adaptive immune response. However, another recent study involving a case of RSV disease in a 15-month-old patient, in which death was due to a vehicular accident rather than the virus, provided evidence of substantial immune infiltrate, including monocytes, T lymphocytes, and neutrophils (72). The controversy over the contribution of immunopathology versus viral cytopathology continues because the natural human host is not amenable to pathogenesis studies and animal models are poor surrogates.

Excessive T-lymphocyte cytotoxicity is one potential mechanism of immune-mediated pathogenesis. Studies of mice showed that the T-cell response helps resolve RSV infection but can make a substantial contribution to disease. For exam-

ple, depletion of $CD4^+$ or $CD8^+$ T cells reduced disease, and depletion of both resulted in long-term infection without illness (53). In a patient with severe combined immunodeficiency and a high level of persistent RSV shedding, T-cell reconstitution dramatically reduced viral shedding but also resulted in a dramatic increase in pulmonary disease (35). Conversely, there also is evidence arguing against a prominent role of T-lymphocyte cytotoxicity in RSV pathogenesis. Among infants with immunodeficiencies, those with cell-mediated deficits have more difficulty controlling the virus and have more severe outcomes, suggesting that the net effect of T cells is protective rather than pathogenic (43). As already noted, one study of lung autopsy specimens provided evidence of a deficient rather than overly robust $CD8^+$ T-cell response associated with fatal RSV disease (159), although a substantial response was observed in a second study (72). In infants hospitalized for RSV bronchiolitis, RSV-specific CTLs were not detected until at least 6 days later (20) and therefore did not correlate temporally with severe disease. Thus, T cells are important for clearing RSV infection, but their contribution to pathogenesis may depend on the situation.

A role for Th2-biased responses in RSV pathogenesis was suggested by (i) the Th2-mediated disease associated with FI-RSV discussed above, (ii) the Th2 bias of the young infant (discussed later), in whom severe disease is more frequent, and (iii) the association of Th2 responses with asthma, which involves small airway constriction, mucus plugging, and wheezing similar to patterns seen with RSV disease. A number of clinical studies have documented elevated ratios of Th2/Th1 cytokines or their mRNAs, measured in nasal secretions or in stimulated or nonstimulated peripheral blood mononuclear cells, in association with severe pediatric RSV disease (80, 92, 93, 131). In some cases, the overall Th response actually was decreased, but the relative Th2 component was increased. In addition, two studies demonstrated a positive association between RSV disease and a genetic polymorphism in the Th2 cytokine IL-4 gene that increases gene expression (69). Conversely, other groups have reported a Th1-biased response or mixed responses associated with severe pediatric RSV disease (13, 47, 107). Thus, there is suggestive but inconsistent evidence of a link between an increased Th2/Th1 ratio and RSV pathogenesis. In some studies, patients with severe disease fell into subgroups with respect to Th responses, suggestive of substantial host variability (80, 107).

The Th2 cytokines IL-4 and IL-13 promote isotype switching to IgE. IgE is bound by receptors on mast cells and basophils and, upon contact with antigen, induces cell activation and the release of mediators, including histamine and leukotrienes. These can mediate neural, vascular, and muscular responses, including rhinorrhoea, cough, and wheeze, which are disease signs associated with RSV. Indeed, some studies have found persistent increased levels of free RSV-specific IgE and histamine in secretions of infants experiencing an RSV infection with wheezing (158). However, other studies have not confirmed these findings (28), and the association of IgE to RSV disease remains unclear.

Eosinophils can be involved in either Th2-mediated or inflammatory responses (see below) and have been suspected to have a role in RSV pathogenesis based on several observations. Increased numbers of eosinophils were reported in autopsy lung tissues from two infants who died of enhanced RSV disease subsequent to vaccination with FI-RSV, although a reanalysis of these specimens indicated that they were a minor population (123). Pulmonary eosinophilia is observed in connection with Th2 responses to RSV antigens in BALB/c mice, as already noted. Also, increased levels of eosinophil degranulation proteins were found in respiratory secretions from patients with severe cases of RSV disease (46, 64). The prominent association of eosinophils in asthma also makes them of particular interest. Eosinophils are recruited by Th2 or inflammatory chemoattractants, including IL-5, eotaxin/CCL11, and RANTES, and are activated to release cytotoxic proteins and antiviral RNase as well as Th2 cytokines and inflammatory chemokines and cytokines. However, the prevalence of eosinophils among airway leukocytes from RSV-infected infants, 1 to 3%, was low and approximately the same as for influenza virus, and thus does not seem to represent a prominent or unique feature of RSV pathogenesis (101, 143). Furthermore, the net effects of eosinophils are not necessarily pathogenic: hypereosinophilia in a transgenic mouse that overexpresses IL-5 was associated with protective and disease-sparing effects against RSV rather than increased disease (118). Thus, while eosinophilia sometimes is prominent in animal models of RSV pathogenesis, its contribution to authentic human disease is unclear.

Overly robust inflammatory responses also have been suggested to contribute to RSV pathogenesis. These initiate when the virus interacts with respiratory epithelial cells and macrophages and is detected by TLRs and other pattern-recognition proteins, leading to upregulation of the expression of inflammatory factors, such as chemokines and surface proteins involved in cell interactions. The further activation of NF-κB by the RSV F and NS2 proteins (described later) likely augments the response (90, 141). This leads to the influx and activation of leukocytes, which add to the production of inflammatory factors and can help resolve infection but also contribute to pathogenesis through tissue damage and other effects. Clinical studies have documented increased expression of inflammatory mediators or their mRNAs in respiratory secretions of infants and children hospitalized for RSV disease compared to controls, including IL-6, tumor necrosis factor α, IL-8, RANTES, macrophage inflammatory protein 1α/CCL3, eotaxin, and monocyte chemotactic protein 1/CCL2, among others (47, 64, 100). As is typical for acute inflammation, neutrophils are the predominant airway leukocyte in infants with RSV bronchiolitis, accounting for 84% or more of the cells, compared to 66% for influenza virus (101, 143). IL-8 is the major chemoattractant for neutrophils. Its concentration in respiratory secretions is elevated in infants with severe RSV disease (1, 64) and in some reports appeared to be somewhat increased for RSV versus other respiratory virus infections (48, 50). Activation of neutrophils occurs in response to inflammatory mediators and possibly to RSV itself and results in the release of cytotoxic enzymes in addition to inflammatory cytokines and chemokines (1). Neutrophils presumably can have antiviral activity due to the destruction of RSV-infected cells, to which they may be attracted by the virus-induced expression of surface adhesion molecules. However, the extent to which RSV neutrophilia and other aspects of the inflammatory response are

protective versus pathogenic, and whether this is particular to RSV compared to other respiratory viruses, is unclear.

As noted, genetic polymorphisms that increase IL-8 and CCR5 expression have been associated with increased RSV disease, consistent with a role of inflammatory chemokines in RSV disease (69). However, a strong inflammatory response does not seem to be essential for the development of viral respiratory tract disease: in one study, the level of inflammatory cytokines and cytokines in nasal wash samples from infected infants was substantially lower for HMPV than for RSV despite similar disease signs (91). In most clinical studies, anti-inflammatory therapy (oral or inhaled corticosteroid) has not provided significant improvement of short- or long-term outcomes of RSV infection (14).

Thus, a number of host immune factors involved in restricting and clearing the virus likely also contribute to pathogenesis, at least under some conditions. However, it is unclear whether one or more factors are particularly responsible for RSV disease, and whether this is different for RSV than for other respiratory viruses.

PATHOGENIC FACTORS DETERMINED MAINLY BY THE VIRUS

High infectivity. RSV is one of the most contagious human pathogens, comparable to measles virus. In prospective studies, the natural introduction of RSV into a day-care setting resulted in infection of more than 90% of infants and children (75). RSV is readily introduced and spreads with ease in hospitals, nursing homes, families, and other close-contact settings (61). High infectivity contributes to yearly epidemics and to the high frequency of reinfection.

RSV is not highly cytopathic or invasive. In an in vitro model of a polarized, mucociliary airway epithelium, RSV preferentially infected and was shed from the ciliated cells of the apical surface (165). This is consistent with clinical histopathology findings, as already noted. Ciliary action was disrupted, and infected cells were shed and replaced over several weeks without gross histological effect despite the ongoing infection. No cell-to-cell fusion was detected, probably because the F protein was expressed on the apical surface and had minimal contact with neighboring cells. In contrast, control cultures infected with influenza A virus were rapidly destroyed within 2 days, and all cell types within this complex tissue appeared to be susceptible to infection. Thus, RSV appears to be inherently less cytopathic and less invasive within the epithelium than influenza A virus and, instead, is more similar to HPIV3 (164). The viral replication cycle in vitro is relatively long (30 to 48 h). Total cellular DNA, RNA, and protein synthesis are reduced only modestly and only late in the cycle, probably through indirect effects.

Limited antigenic and strain diversity. RSV has a single serotype with two major antigenic subgroups (A and B) (21). Some studies have described subgroup A strains as being associated with increased disease severity, but other studies have not confirmed this difference in virulence. Recently, a subgroup A strain called line 19 was shown to induce increased airway hypersensitivity and goblet cell expansion in mice compared to the related A2 prototype strain, providing the first clear example (albeit in mice) of a strain-specific difference in RSV illness (96). So far, however, strain differences do not seem to play an important role in disease variability in humans.

Between the two subgroups, G is the most divergent protein, with 53% amino acid sequence identity and 1% to 7% antigenic relatedness between subgroups (21). However, because the other neutralization antigen, F, is less divergent (90% amino acid sequence identity and 50% antigenic identity), the two subgroups exhibit only a three- to fourfold difference in reciprocal cross-neutralization in vitro. The subgroup difference has been estimated to confer a fourfold advantage in reinfection (161). Epidemics typically involve multiple circulating strains in which subgroup A and B may alternate in predominance every 1 or 2 years. Yearly variation in the virus population in any locale mainly involves changes in the mixture of circulating strains rather than progressive antigenic drift. The two antigenic subgroups are estimated to have diverged 350 years ago (167) and, as already noted, still retain a high level of antigenic relatedness.

Infection very early in life. RSV infects patients earlier in life with greater consequences than other respiratory viruses. Rhinoviruses, influenza viruses, HPIVs, and HMPV commonly infect children <6 months of age (55), but RSV causes more frequent and severe infections at an earlier age. Rhinovirus infection in particular is common during the first year of life and is associated with wheezing, much like RSV, but has a much lower incidence in infants <6 months of age (105). In contrast, the peak of hospitalization for RSV disease is at the remarkably young age of 2 months.

The ability to infect infants very early in life increases the impact of RSV. Very young infants are less tolerant of severe infection than older individuals, in part because they have airways that are narrower in diameter and thus more susceptible to obstruction, a prominent feature of RSV disease. Immune responses in general are lower in magnitude and presumably less effective in young infants than in older children or adults. For example, in a clinical trial of a live-attenuated intranasal RSV vaccine, 100% of seronegative vaccinees ages 6 months or more achieved a fourfold-or-greater increase in RSV-specific serum antibodies after one vaccine dose compared to 44% of infants ages 2 to 4 months after two doses (78). Comparable differences are seen for natural infections with wild-type virus. RSV-specific cytotoxic cellular responses to natural infection were detected in less than 40% of infants under 5 months of age compared to 65% of children 6 months to 2 years of age (20). Protective responses against respiratory viruses in infancy also are not long lasting (66, 76).

The reduced immune responses are due to two phenomena: immunosuppression by RSV-specific maternal serum antibodies (26) and immunologic immaturity (2, 87). Antibody-mediated immunosuppression affects mainly the humoral response to RSV (26), and its mechanism is poorly understood. Immunologic immaturity has multiple aspects and affects innate, humoral, and cell-mediated immunity (2, 87). Compared to adults, young infants have a smaller number of lymphocytes in peripheral lymphoid tissues. Neonatal DCs have been shown to be less efficient than adult cells in supporting T-cell proliferation in response to antigen stimulation in vitro and to secrete fewer cytokines in response to RSV (87). They also are deficient in the production of IL-12 and in inducing IFN-γ. In

addition, infants exhibit a reduced frequency of somatic mutation in the development of their antibody response and a diminished capacity for class switching (155, 156). While the impact of antibody-mediated immunosuppression on RSV-specific antibody responses is well documented and is substantial (26), the impact of immunologic immaturity is less well defined.

There is a lingering Th2 bias in responses bias during the neonatal period; during pregnancy, this helps block Th1-mediated immune rejection of the fetus (2, 121). Infection with RSV during the first 3 months of life has been shown to induce a Th2-biased response compared to infection of older infants, based on an analysis of cytokines in respiratory secretions (88). Interestingly, this also was true of infants infected with HPIV or influenza virus and thus may be more related to host age rather than virus (88), but the overall impact of this effect is likely to be greater for RSV, since it infects patients with a greater frequency at this young age. Infection in a Th2-biased environment has the potential to affect the quality of the primary and recall responses. Infection of neonatal mice with RSV was associated with reduced and delayed expression of IFN-γ during primary infection and increased disease and Th2-associated cytokines upon reinfection compared with older animals (27). This result indicates that the age at first infection can have consequences on the host primary and recall responses (27). The majority of human infants do not have severe disease or lingering clinical manifestations of RSV infection, and there is no evidence of enhanced disease upon reinfection. However, there may be subtle effects of immunological imprinting of early infection in genetically susceptible individuals. The Th2 environment also has the potential to promote sensitization to bystander environmental antigens, as has been demonstrated for mice (116). Conversely, the "hygiene hypothesis" postulates that (nonsevere) infections early in life by RSV and other pathogens can stimulate the maturing immune system toward adult-like Th1 responses, but whether this occurs with respiratory viruses in general and with RSV in particular remains a topic of investigation (44).

In addition to immune maturation, infancy is a time of rapid lung maturation involving pulmonary alveolar expansion and airway remodeling to accommodate growth (49). Whether virus-induced damage and overexpression of cellular factors during this period can have long-term effects on lung function or the immune response in some individuals is largely unknown. This might depend on infection occurring in a genetically susceptible infant at a critical time in the development of the immune system or the lung (49, 98). As one example, RSV infection of rats was shown to increase the pulmonary expression of nerve growth factor (NGF) and its receptors, an effect that was substantially greater (and well beyond normal levels) in weanling versus older animals (68). NGF upregulates the expression of the tachykinin substance P and its receptor, which augment inflammation and airway hypersensitivity. The age-specific aspect of this effect might contribute to greater disease in the young. Overexpression of these neurogenic factors at critical times might potentially have long-term consequences. Increases in NGF and another neurotropin also have been found in cells recovered from the lower airways of infants with severe RSV disease versus controls (149).

Reinfection. RSV is able to reinfect throughout life, and even in the same epidemic season, despite limited antigenic variation (59). A 10-year prospective day-care study found that the majority of children were infected in each of the first 3 years of life (66). Frequent reinfection also is seen in adults that are exposed to the virus: during a typical RSV season, 25 to 50% of health-care workers are reinfected, and family members of sick children are readily reinfected. The annual incidence of natural RSV infection in healthy adults not selected for high exposure to the virus is estimated at 5 to 10% (39, 59). Experimental infection studies have documented considerable variability in the susceptibility of adults to reinfection (60). Higher RSV-specific serum and nasal wash antibody titers tend to correlate with greater resistance to reinfection, although a substantial proportion of subjects with high titers can be reinfected (60, 106).

RSV reinfection usually is associated with reduced disease compared with primary infection, although even in healthy adults, 84% of infections in a prospective study were symptomatic and 26% had lower respiratory tract involvement (59). Reinfection in infants, in the elderly, and in severely immunosuppressed individuals can result in severe disease. Importantly, the ability of RSV to reinfect maintains its presence in the population and increases its access to susceptible individuals of all ages. The ability of RSV to reinfect without considerable antigenic change is in sharp contrast with the ability of influenza A virus to reinfect, which is strongly dependent on antigenic drift or shift. However, reinfection is not unique to RSV among respiratory viruses: the HPIVs also can reinfect without antigenic variation, although this does not occur as frequently as with RSV (57, 112).

Tissue tropism. The tropism of RSV for the superficial cells of the respiratory tract reduces the effectiveness of host immunity. As noted, local IgA responses can be short-lived. Serum antibodies (maternal, prophylactic, or postinfection) gain access to the respiratory lumen by the inefficient process of transudation, which results in an estimated 80- to 160-fold gradient between serum and the respiratory lumen (124). A further 10-fold increase in serum antibodies is required for protection in the nasal cavity compared to the lungs, which likely reflects a difference in the efficiency of transudation into these compartments (137). Thus, a high serum antibody titer is required to confer protection. These factors leave the upper respiratory tract particularly vulnerable to infection. However, that should be the case for any respiratory virus and thus does not alone account for the unusually high infectibility and reinfectibility of RSV.

It was recently shown that virus-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) became functionally impaired in the production of antiviral cytokines and in cytolytic capacity after recruitment to the lungs in virus-infected mice, an effect that was observed for RSV, influenza virus, and simian virus 5 (153). Thus, this impairment was tissue specific rather than virus specific, and it did not require CTL contact with a specific viral antigen. This may be a host mechanism to prevent excessive pulmonary inflammation and damage, but it has the potential to inappropriately reduce CTL activity against respiratory viruses.

Tropism to the superficial luminal cells of the airway, low invasiveness, and inhibition of apoptosis (by the NS1, NS2, and

SH proteins; see below) might each contribute to delay the presentation of RSV antigens to the immune system. Lack of virus-mediated destruction of the full epithelium would maintain the steep gradient of transfer of antibodies from the serum.

NS1 and NS2 proteins. Among the respiratory viruses, RSV is one of the most effective in blocking synthesis of host type I IFN in infected individuals (58). RSV has two IFN antagonists, NS1 and NS2, which are encoded from promoter-proximal genes to ensure high expression. These inhibit the induction of IFN-α/β by blocking the activation of IFN regulatory factor 3 and by inhibiting type I IFN-induced signaling through the JAK/STAT pathway, which otherwise amplifies the IFN response, upregulates IFN-stimulated genes, and establishes an antiviral state (95, 141). One or both of the NS proteins target STAT2 for proteasomal degradation, thus blocking signaling from type I IFN (34, 95). Infection of STAT1 knockout mice with RSV resulted in a Th2-biased response and increased lung pathology compared to that of wild-type animals despite a similar level of replication (32). This suggests that, in the natural host, antagonism of the IFN response by NS1 and NS2 may increase the Th2/Th1 balance.

The level of activation of NF-κB in epithelial cells in response to RSV infection was greatly reduced by deleting the NS2 gene (141). How the expression of NS2 increases the activation of this transcription factor is unknown, although it might be related to the recent finding that the expression of NS1 and/or NS2 activates the phosphoinositide 3-kinase (PI3K) pathway (11). This is an intracellular signaling pathway, orchestrated by increased phosphorylation of the phospholipid phosphatidylinositol on the inner face of the plasma membrane, which enhances cell survival, among other effects. Suppression of the expression of NS1 and/or NS2 by short interfering RNAs or by viral gene deletion suppressed activation of the PI3K pathway and resulted in accelerated apoptosis of RSV-infected cells and a reduction in virus yield (11). By activating the PI3K pathway, NS1 and NS2 increased the survival time of the infected cell and increased the yield of progeny virus.

Recombinant BRSV lacking the NS1 and NS2 proteins was highly attenuated but more immunogenic in bovines than the wild-type virus (152). Improved immunogenicity might be due to increased IFN signaling creating an adjuvant effect or more rapid apoptosis leading to efficient cross-priming and antigen presentation. Increased IFN signaling and apoptosis also would attenuate viral replication.

G protein. The G protein has a number of features with roles in immune evasion and attenuation of the immune response, and these roles continue to be elucidated. G is an unusual viral neutralization antigen in that very few individual G-specific monoclonal antibodies efficiently neutralize infectivity; neutralization requires multiple antibodies (103). The ectodomain of G is extensively decorated with carbohydrate side chains and contains many more potential acceptor sites for O-linked sugars. Heterogeneity in the placement and structure of the sugar side chains could introduce antigenic diversity by creating subpopulations that differ in the efficiency of binding by particular antibodies. In addition, the extensive sheath of sugar side chains probably helps shield the polypeptide backbone from immune recognition.

As another immune evasion mechanism, the secreted form of G (sG) was recently shown to act as a decoy that helps shield virus from neutralization by RSV-specific antibodies (A. Bukreyev, L. Yang, J. Fricke, B. R. Murphy, and P. L. Collins, unpublished data). In cell culture, sG is secreted rapidly and in considerable quantity such that, at 24 h postinfection, it accounts for 80% of the released G protein compared to 20% contained in progeny virus (67). This early, abundant expression suggests that, in vivo, sG might flood the local environment of the infected cell and saturate RSV-specific antibodies. This provides the first description of a mechanism by which RSV might evade neutralizing antibodies more efficiently than other respiratory viruses.

The cystine noose in the G ectodomain contains a CX3C motif embedded in a region that has limited sequence relatedness with the CX3C chemokine fractalkine (151). Fractalkine/CX3CL1 is produced in secreted and membrane-bound forms that function as chemoattractant and cell adhesion molecules to mediate the influx of $CX3CR1^+$ leukocytes, which include subsets of NK cells and $CD4^+$ and $CD8^+$ T lymphocytes. RSV G, which also is produced in secreted and membrane-bound forms, was shown to have fractalkine-like chemoattractant activity in vitro (151). In vivo, there is evidence that G has the effect of a fractalkine antagonist. In mice, infection with a mutant RSV in which the CX3C motif had been ablated by a single-amino-acid substitution, or one that lacked G protein altogether, was associated with a substantial increase in pulmonary NK cells and $CD4^+$ and $CD8^+$ T cells compared to wild-type RSV (63). Furthermore, when RSV-specific responses were evaluated, ablation of the CX3C motif in G was associated with a substantial increase in RSV-specific pulmonary $CD8^+$ CTL that were functional in an in vitro cell-killing assay. These data suggest that the RSV G protein substantially reduces recruitment and functionality of pulmonary $CX3CR^+$ leukocytes, including RSV-specific CTLs, presumably by competitively inhibiting the functions of host fractalkine. However, it should be noted that a second study found the opposite effect, namely, that the G protein enhanced the pulmonary $CD8^+$ T-cell response to RSV in mice, an effect that depended on the conserved cystine noose (16). In any event, deletion of the cystine noose and fractalkine domain from RSV had little effect on the efficiency of viral replication in mice, suggesting that their quantitative impact on virus replication might not be great (146).

The G protein also influences the innate response of epithelial cells and antigen-presenting cells to RSV infection. Infection of human epithelial cells and monocytes with an RSV mutant that does not make sG resulted in increased activation of NF-κB and increased expression of inflammatory mediators, such as IL-6, IL-8, and RANTES, compared to infection with wild-type virus (5). This result suggests that sG normally downregulates this host innate response. A second study confirmed that G strongly inhibits the NF-κB-mediated inflammatory response to RSV in human monocytes and showed that this effect required the cystine noose domain (119). Interestingly, G also was found to suppress the inflammatory response to known agonists of TLR2, TLR4, and TLR9 (119). Thus, the G protein appears to act as a general TLR antagonist to downregulate inflammatory responses by a mechanism that is not yet known.

The G glycoprotein influences the pattern of lymphocyte cytokine expression in murine models and could potentially induce similar effects in selected humans. Immunization of BALB/c mice with a recombinant vaccinia virus expressing RSV G induces a Th2 $CD4^+$ T-lymphocyte response that leads to pulmonary eosinophilia following RSV challenge (113). This is the result of a Vβ14-restricted clonal response to a single epitope in G (amino acids 185 to 198) (154). Interestingly, immunization with vaccinia virus expressing sG induced even greater airway eosinophilia postchallenge than vaccinia expressing wild-type G or membrane-anchored G (73). Vaccinia virus expressing sG directly induced IL-5 and IL-13, producing pulmonary eosinophilia, and enhanced mucus production postchallenge, providing another indication that sG alters the host response (73). Whether selected humans have biased responses to G or to other individual RSV proteins is not known but is a potential factor in primary infections associated with allergic inflammation.

Studies with PVM, the murine relative of RSV, provided evidence of an additional role for the G protein in pathogenesis (86). In the respiratory tracts of mice, wild-type PVM replicates efficiently, causing severe disease and death. Virus from which the G gene had been deleted replicated poorly and did not cause disease. Virus from which the first 34 amino acids of G (comprising the complete cytoplasmic domain) had been deleted replicated as efficiently as the wild-type virus but, surprisingly, was nonpathogenic at comparable doses. None of the viruses produced a sG, and PVM G does not contain a CX3C motif, indicating that these factors were not involved. This is the first report of a disease-attenuating mutation in a pneumovirus that cannot be explained simply on the basis of a reduction in viral load. It may be that the cytoplasmic tail itself is an important factor in virulence, perhaps initiating intracellular signaling pathways that remain to be identified.

F protein. Even though the F protein has two cleavage sites, activation appears to be readily achieved by ubiquitous cellular proteases. There is no evidence that cleavage activation is a limiting factor in RSV tissue tropism or pathogenesis, in contrast to well-known models, such as the paramyxovirus Newcastle disease virus and avian influenza A virus. In the case of BRSV, the p27 fragment that is released by cleavage at the two sites was found to be related by sequence and function to tachykinins, a family of neuropeptides that promote airway hypersensitivity and inflammation (166). However, there appears to be no similarity between the p27 fragment of human RSV and tachykinins.

The RSV F protein has been shown to bind to, and initiate signaling through, TLR4 and its accessory protein CD14 (90). This effect presumably would oppose that of the TLR4 antagonism noted above for the G protein, suggesting that there is a balance in RSV infection. TLR4/CD14 also binds lipopolysaccharide and surfactant protein A, among other ligands, and is abundant on monocytes and DCs. Human airway epithelial cell lines normally express very low levels of TLR4, but its expression is increased in response to RSV infection, which would provide for amplification of the inflammatory response to infection (108). Interestingly, contact between a number of different respiratory viruses or bacteria and the mouse epithelium results in rapid inhibition of Na^+ transport, resulting in apical fluid accumulation (89). This might be an epithelial mechanism to dilute and remove irritants but might also contribute to virus spread and disease. In the case of RSV, but not the other pathogens, this phenomenon appears to be initiated by ligation of the F protein with TLR4.

The overall effect of TLR4 on RSV pathogenesis is unclear. Silencing the TLR4 gene in mice appeared to have no discernible effect on viral replication, disease, or the host response during infection with RSV or with its murine counterpart PVM (33, 37). In humans, several studies have investigated a possible link between pediatric RSV disease and two coding polymorphisms in TLR4 that are associated with TLR4 hyporesponsiveness and increased susceptibility to bacterial infection (6, 115, 126, 144). Tal et al. found that these two polymorphisms indeed were associated more frequently with RSV bronchiolitis than with controls (144), consistent with a protective role for TLR4. However, Paulus et al. did not confirm this association (115), and Puthothu et al. provided evidence of an effect in the opposite direction, namely, that the same TLR4 polymorphisms might be associated with reduced rather than increased RSV disease (126). Interestingly, in a recent study, a cohort of largely premature, high-risk infants with confirmed RSV disease was found to have remarkably high frequencies of the two TLR4 polymorphisms (6). The authors suggest that TLR4 hyporesponsiveness may be associated with increased susceptibility to both premature birth (perhaps due to in utero infection) and RSV disease.

An analysis of the antibody response to RSV infection in humans provided evidence that the F protein is presented to the immune system in two versions that induce antibody responses of comparable magnitudes, namely, a mature form, as found in virions, and incorrectly folded forms that lack important neutralization epitopes (132). The latter might represent immature protein released from lysed cells or denatured mature protein, possibly related to the noted physical instability of RSV. The substantial antibody response against incorrectly folded forms raises the possibility that they might have the effect of diverting and reducing immune recognition of conformationally correct protein. Whether this is unique to RSV among respiratory viruses is not known.

SH protein. The SH protein does not seem to play a major quantitative role in RSV replication and pathogenesis, since deletion of SH from recombinant RSV had little effect on virus production in vitro and resulted in only a small decrease in replication in chimpanzees. Furthermore, the deletion of SH from an attenuated vaccine candidate did not confer a further attenuating effect in seronegative children (78). Molecular modeling suggests that the SH protein may be an ion channel-forming viroporin (84). Viroporins have been identified for a number of viruses and play roles in virus assembly and release as well as in pathogenesis and cytotoxicity. SH has been shown to form pentamers and, when expressed in bacteria, to alter membrane permeability (22, 117). Recent evidence indicates that the expression of SH delays apoptosis, probably by inhibiting signaling from tumor necrosis factor α (45). Combined with the antiapoptotic effect of the NS proteins, this might increase virus replication by prolonging cell survival and might also delay antigen processing through cross-priming.

Effects on macrophages and DCs. Alveolar macrophages represent one of the first lines of host defense against respiratory infection and are active in phagocytosis, secretion of mi-

crobicides and inflammatory cytokines, and antigen presentation. Myeloid DCs serve as major antigen-presenting cells, and plasmacytoid DCs are a major source of IFN-α/β. Myeloid and plasmacytoid DCs, and indeed all circulating immune cells, are recruited to the respiratory mucosa during infection with RSV and other respiratory viruses, and there is evidence of RSV infection of DCs in vivo (Fig. 2) (50, 72). In vitro, RSV can infect alveolar macrophages and DCs (from cord blood or peripheral blood) at efficiencies of up to 50% and 30%, respectively, although the efficiency of infection of DCs usually is much lower (7, 56, 104). Infection or uptake can induce DC maturation based on changes in the expression of surface markers and in the secretion of chemokines and cytokines. For the most part, this depends on viral infectivity.

RSV appears to interfere with the functions of macrophages and DCs in a number of ways. First, RSV-infected monocyte-derived myeloid DCs make very little IFN-α/β relative to that made by HMPV-infected cells (56), and RSV blocks the induction of plasmacytoid DC maturation and IFN production in response to TLR7 and TLR9 agonists (134). Second, compared to influenza virus and HPIV3, RSV induces a somewhat different spectrum of cytokines from cord blood macrophages and DCs; specifically, there was a reduced level of IL-12 and a greater secretion of IL-10, IL-11, and prostaglandin E2, which might suppress T-cell activation and favor an increased Th2/Th1 balance (7, 114). Third, RSV infection of cord blood or monocyte-derived myeloid DCs decreased their capacity to activate $CD4^+$ T cells (7, 29). Surprisingly, part of this effect was mediated by IFN-α and -λ (19). Fourth, DCs persist and even increase in number at the respiratory mucosa of infants for up to 8 weeks following the resolution of disease, a finding that also has been observed with the mouse model (10, 50). This raises the possibility of a defect in the trafficking of DCs from the lung to lymphoid tissue in response to RSV infection. These observations suggest that RSV alters DC biology to reduce the influence of IFNs, shift the Th2/Th1 balance, and limit the maturation and possibly the mobility of these important cells. These effects are compounded in the neonate because of the immaturity of the antigen-presenting cells. These effects might alter the immune response quantitatively and qualitatively.

DISCUSSION

RSV is a highly infectious and prevalent virus. More than other respiratory viruses, RSV infection can occur very early in life despite maternal antibodies, and reinfection can readily occur throughout life without significant antigenic change. These capabilities likely involve a number of factors, some of which are not unique to RSV. These include high infectivity, tropism to the superficial respiratory epithelium, low invasiveness, the antibody decoy activity of sG, expression of type I IFN antagonists, expression of fractalkine and TLR antagonists, inhibition of apoptosis by multiple viral proteins, the unusual antigenic properties of G, a modest level of antigenic variability, interference with normal macrophage and DC function, and possible additional deficiencies in the protective immune response. Thus, RSV infects an anatomical site where host defense is reduced in effectiveness and employs factors that blunt the host response and provide for immune evasion. Figure 3 depicts a speculative model of the impact of major host and viral factors in RSV pathogenesis.

The unusual ability of the virus to evade maternal antibodies and efficiently infect infants early in life is a major factor in RSV disease. The young infant is less able to tolerate a severe respiratory infection due to its small size and narrow airways. This is aggravated by the tropism of RSV to the small bronchioles, which are particularly prone to obstruction. The young infant also is less able to control infection because of immunologic immaturity and the immunosuppressive effect of maternal antibodies. RSV colludes with the inherent vulnerabilities of the neonatal immune system to induce an immune response that is inadequate and short-lived, at least early in life, with a particularly deficient IFN component. It seems reasonable to suspect that neonatal infection of certain individuals may, depending on timing and genetic background, meditate long-term pathogenic changes to the developing lung and host response. Such an effect may not necessarily be unique to RSV, but RSV is more able than other respiratory viruses to infect the vulnerable neonate. It is possible that the immunological imprint of this first infection is related to the lifelong susceptibility to RSV reinfection.

There is a broad heterogeneity of RSV disease in primary infection, encompassing upper respiratory tract symptoms, lower respiratory tract symptoms of varying levels of severity and with or without wheezing, death in rare cases, airway reactivity that can last through childhood, and possible allergic hypersensitivity. This does not seem to involve substantial differences in virulence among circulating strains of RSV, but host factors certainly are involved. As already noted, these include conspicuous factors affecting the ability to endure and control a respiratory infection, such as bronchopulmonary dysplasia and immunodeficiency. Other likely factors of a more subtle nature include a predisposition for airway hypersensitivity, a predilection for insufficient (e.g., reduced function of TLR4 or surfactants) or exaggerated (e.g., increased expression of IL-4 or IL-8) immune responses, and unusually narrow airways (98). The ongoing identification of genetic polymorphisms associated with increased (or decreased) risk of severe RSV disease illustrates that numerous host factors contribute to the variability in host susceptibility and pathogenesis. Eventually, genetic polymorphism analysis may help identify the risk of serious disease.

The relative contribution of viral versus various host factors to RSV pathogenesis remains controversial. Major proposed features include direct viral cytopathology, exaggerated CTL responses, imbalanced Th2/Th1 responses, exaggerated inflammatory responses, and insufficient or altered responses due to young age or viral factors. Various studies have tended to emphasize selected individual factors. Pathogenesis may indeed be simple in some cases: for example, in some cases, the virus might simply outrace host defense and quickly overwhelm the young infant, such that viral cytopathology is predominant (159). In most cases, however, there may not be a single predominant factor. Instead, there may be different relative contributions from the various interacting viral and host factors that will depend on the speed and magnitude of viral replication, the effectiveness of the host response, underlying predispositions toward aberrant, exaggerated, or deficient aspects of the host response, maturational state, and other factors. Thus,

pathogenesis usually is multifactorial and varied (Fig. 3). A better understanding of the viral and host determinants of RSV disease will be important for designing vaccines and therapeutic agents. In particular, defining the consequences of primary infection at a time of developmental and immunological immaturity may be a key to the riddle of RSV pathogenesis.

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